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Research Article

# Morphological and multi-gene phylogenetic analyses reveal *Nigrellomyces* gen. nov. and one new species in Pleurotheciaceae from China

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## Abstract

*Nigrellomyces* is introduced herein as a new genus to accommodate a novel asexual ascomycete, *Nigrellomyces aquaticus*, isolated from submerged decaying wood in freshwater habitats in Guizhou Province, China. Phylogenetic analyses based on a combined dataset of ITS, LSU, SSU, and *rpb*2 sequence data robustly place *Nigrellomyces* within the family Pleurotheciaceae (Pleurotheciales, Savoryellomycetidae), with strong statistical support. The genus is currently known only from its asexual morph, which is characterized by macronematous, mononematous, erect, unbranched, septate conidiophores; polyblastic, integrated, terminal or intercalary, sympodial proliferations; denticulate conidiogenous cells with swollen apices; and acrogenous, subglobose to globose, ovoid to obovoid, or ellipsoidal, aseptate, guttulate conidia. Morphological comparisons reveal that *Nigrellomyces* displays a cordana-like conidiogenesis that is distinct from other genera in Pleurotheciaceae, which typically exhibit acrodictys-, helicoön-, monodictys-, or dactylaria-like asexual morphs. This study provides a comprehensive morphological description, illustrations, and comparative interpretation of the new taxon. An updated phylogenetic backbone tree of Pleurotheciaceae is also presented, improving resolution of intergeneric relationships within the family. The discovery of *N. aquaticus* not only expands the taxonomic framework of Pleurotheciaceae but also contributes to a deeper understanding of the species richness, ecological roles, and biogeographic distribution of lignicolous freshwater fungi in Southwest China, particularly in the underexplored region of Guizhou Province.

**Key words:** Asexual morph, lignicolous freshwater fungi, phylogeny, Sordariomycetes, taxonomy

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## Introduction

Lignicolous freshwater fungi are a specialized group of fungi that colonize submerged or partially submerged woody substrates across a wide range of freshwater habitats, including streams, rivers, lakes, and reservoirs (Luo et al. 2004; Hyde et al. 2016; Calabon et al. 2022; Calabon et al. 2023). These fungi play an essential ecological role in the degradation of lignocellulosic materials, contributing significantly to the breakdown and recycling of

organic matter. Through this decomposition process, they facilitate nutrient release and cycling, thus maintaining the ecological balance and supporting the biodiversity of freshwater ecosystems (Yuen et al. 1998; Bucher et al. 2004; Hyde et al. 2016; Calabon et al. 2023).

In recent decades, Asia has emerged as a global hotspot for lignicolous freshwater fungal research, with numerous studies focusing on the diversity and taxonomy of these fungi (Hyde et al. 2016; Su et al. 2016; Luo et al. 2019; Dong et al. 2020; Bao et al. 2021, 2023; Calabon et al. 2022, 2023; Shen et al. 2022b, 2024; Yang et al. 2023; Wang et al. 2024b; Xu et al. 2025b). In-depth investigations have been conducted, particularly in regions such as Guizhou, Hong Kong, Taiwan, and Yunnan in China, and Chiang Rai and Chiang Mai in Thailand. These regions have yielded a remarkable number of newly described taxa and have significantly expanded our understanding of the diversity and distribution of lignicolous freshwater fungi in tropical and subtropical habitats (Zhang et al. 2011; Jones et al. 2014; Hyde et al. 2016, 2021; Su et al. 2016; Bao et al. 2018, 2019, 2020, 2021, 2023; Luo et al. 2018b, 2019; Dong et al. 2020, 2021a, 2021b; Calabon et al. 2021, 2022, 2023; Shen et al. 2022a, 2022b, 2024; Xu et al. 2023, 2024b, 2025a, 2025b; Yang et al. 2023; Wang et al. 2024a, 2024b; Xiao et al. 2025). Among them, members of the Dothideomycetes and Sordariomycetes dominate lignicolous freshwater fungal communities, with ongoing research continuously revealing new species and higher-order lineages within these two classes (Su et al. 2016; Luo et al. 2019; Dong et al. 2020; Hyde et al. 2021; Calabon et al. 2022). Notably, the families Distoseptisporaceae, Fuscosporellaceae, Pleurotheciaceae, Savoryellaceae, Junewangiaceae, and Sporidesmiaceae of the Sordariomycetes constitute some of the most dominant and diverse groups of lignicolous freshwater fungi, encompassing many major taxa (Luo et al. 2019; Yang et al. 2023; Wang et al. 2024a, 2025; Xu et al. 2025b).

The family Pleurotheciaceae was established by Réblová et al. (2016), with *Pleurothecium* designated as the type genus. This family encompasses a diverse assemblage of fungi, including several species that inhabit submerged woody substrates in freshwater habitats (Réblová et al. 2016; Luo et al. 2018a; Dong et al. 2021b; Shi et al. 2021; Bao et al. 2022; Huang et al. 2022; Wang et al. 2024a; Xu et al. 2024a, 2025a). Both sexual and asexual morphs of Pleurotheciaceae have been reported, with the sexual morphs characterized by dark, papillate, perithecial, astromatic, immersed to superficial ascomata, unitunicate asci with a distinct non-amyloid apical annulus, and fusiform to ellipsoidal, septate, hyaline ascospores (Réblová et al. 2016; Luo et al. 2018a; Hyde et al. 2020). The asexual morphs of Pleurotheciaceae include species with diverse morphologies, comprising acrodictys-like (Yanna and Hyde 2002; Sadowski et al. 2012), helicoön-like (Dayarathne et al. 2019; Réblová et al. 2020), monodictys-like (Hyde et al. 2020), and dactylaria-like taxa (Réblová et al. 2016; Luo et al. 2018a).

In the latest update of "The 2024 Outline of Fungi and Fungus-like Taxa" compiled by Hyde et al. (2024), a total of 15 genera are accepted within the family Pleurotheciaceae, including *Adelosphaeria* Réblová, *Anapleurothecium* Hern.-Restr., R.F. Castañeda & Gené, *Coleodictyospora* Charles, *Dematipyriforma* L. Yan Sun, Hai Y. Li, Xiang Sun & L.D. Guo *Helicoascotaiwania* Dayar., Maharachch. & K.D. Hyde, *Melanotrigonum* Réblová, *Monotosporella* S. Hughes, *Neomonodictys*

Y.Z. Lu, C.G. Lin & K.D. Hyde *Phaeoisaria* Höhn., *Phragmocephala* E.W. Mason & S. Hughes, *Pleurotheciella* Réblová, Seifert & J. Fourn., *Pleurothecium* Höhn., *Pseudosaprodesmium* X.G. Tian, K.D. Hyde & Tibpromma, *Saprodesmium* W. Dong & Doilom, and *Sterigmatobotrys* Oudem. The taxonomic placement of certain genera, particularly *Rhexoacrodictys*, has remained controversial. Initially, *Rhexoacrodictys* W.A. Baker & Morgan-Jones was placed in the order Savoryellales by Xia et al. (2017); subsequent molecular phylogenetic analyses by Luo et al. (2019) transferred *Rhexoacrodictys* to Pleurotheciales. Subsequent phylogenetic studies have confirmed the findings of Luo et al. (2019), providing robust support for the reassignment of *Rhexoacrodictys* (Dong et al. 2021b; Shi et al. 2021; Bao et al. 2022; Huang et al. 2022; Wang et al. 2024a, 2025).

Despite "The 2024 Outline of Fungi and Fungus-like Taxa" listing this genus under Savoryellaceae (Savoryellales), current phylogenetic evidence clearly indicates that *Rhexoacrodictys* is best placed in Pleurotheciaceae (Pleurotheciales), and its classification within this family is now widely accepted (Dong et al. 2021b; Shi et al. 2021; Bao et al. 2022; Huang et al. 2022; Wang et al. 2024a, 2025). Furthermore, *Obliquifusoideum* W. Dong & Doilom was recently included in Pleurotheciaceae by Wang et al. (2024a), based on both morphological characteristics and phylogenetic evidence. In the other study, based on morphological characteristics and DNA sequence data, *Saprodesmium* was treated as a synonym of *Rhexoacrodictys* (Wang et al. 2025). To date, the family Pleurotheciaceae comprises sixteen genera and more than 100 species, which are primarily saprobic on a variety of decaying plant substrates in both freshwater and terrestrial habitats (Réblová et al. 2016; Luo et al. 2018a; Dong et al. 2021b; Shi et al. 2021; Bao et al. 2022; Huang et al. 2022; Wang et al. 2024a; Xu et al. 2024a, 2025a).

During a recent investigation of lignicolous freshwater fungi in Guizhou Province, China, a previously undescribed cordana-like species was isolated. Phylogenetic analyses based on a combined dataset of ITS, LSU, SSU, and *rpb*2 gene regions revealed that the new collection formed a well-supported and distinct clade within the family Pleurotheciaceae. Based on a comprehensive assessment integrating detailed morphological observations and multi-locus phylogenetic evidence, we introduce a new genus to accommodate this unique taxon. This study provides a thorough taxonomic treatment of the new taxa, including detailed morphological descriptions, high-quality illustrations, and comparative analyses with closely related taxa. In addition, an updated phylogenetic backbone tree of Pleurotheciaceae is presented, offering insights into the relationships among genera within the family. Our findings contribute not only to the taxonomic richness of lignicolous freshwater fungi in Guizhou Province but also enhance our broader understanding of fungal diversity associated with freshwater habitats in southwest China.

## Materials and methods

### Sample collection and specimen examination

Lignicolous substrates (decaying wood) were collected from a stream in Baiyun District, Guiyang City, Guizhou Province, China. Samples were taken to the laboratory in plastic bags and labeled with collection details, including locality,

habitat, and date (Rathnayaka et al. 2024). Samples were cultured in plastic boxes lined with moistened tissue at room temperature for 1–2 weeks (Yang et al. 2023). The samples were examined using a stereomicroscope (SMZ 745, Nikon, Japan). Micromorphological characters were captured using a Nikon EOS 90D digital camera attached to an ECLIPSE Ni compound microscope (Nikon, Japan). Measurements of conidiophores, conidiogenous cells, and conidia were carried out using the Tarosoft (R) Image Frame Work program.

## Isolation and material deposition

Single-spore isolation was performed following the method described by Senanayake et al. (2020). The germinated conidia were aseptically transferred to fresh potato dextrose agar (PDA; Oxoid, CM0139) and incubated at room temperature for 41 days. Morphological characteristics of the fungal mycelium on PDA, including color, shape, size, margin, and elevation, were documented. Dried fungal specimens were deposited in the Herbarium of Guizhou Academy of Agricultural Sciences (Herb. GZAAS), Guiyang, China. Pure cultures were deposited at the Guizhou Culture Collection (GZCC), Guiyang, China. Descriptions of the new taxa were uploaded to the Faces of Fungi webpage following the guidelines of Jayasiri et al. (2015). The new species were registered in the MycoBank database (https://www.mycobank.org/), and MycoBank numbers were obtained.

## DNA extraction, PCR amplification, and sequencing

Fresh fungal mycelia grown on PDA were scraped using sterilized scalpels. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, China), following the manufacturer's protocol. The primer pairs ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), NS1/NS4 (White et al. 1990), and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were used to amplify the ITS, LSU, SSU, and *rpb*2 regions, respectively. PCR amplification was performed in a 25 µL reaction volume, consisting of 13.5 µL of 2× Taq PCR Master Mix (China; containing Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer), 1 µL of each primer, 1 µL of template DNA, and 8.5 µL of $ddH_2O$. The polymerase chain reaction (PCR) conditions employed were in accordance with the reaction conditions outlined by Wang et al. (2024a). The PCR products were purified and sequenced by Sangon Biotech (Shanghai, China) Co., Ltd.

## Phylogenetic analyses

BioEdit v. 7.0.5.3 (Hall 1999) and SeqMan v. 7.0.0 (Swindell and Plasterer 1997) were used to check and assemble the newly generated sequences. Sequences obtained in this study (Table 1) were deposited in the NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments for each locus dataset were performed using MAFFT v.7.473 (https://mafft.cbrc.jp/alignment/server/, Katoh et al. 2019) and visually inspected in AliView (Larsson 2014). The LSU and ITS alignments were trimmed using trimAl v1.2rev59 (Capella-Gutiérrez et al. 2009) and subsequently merged using SequenceMatrix v1.7.8 (Vaidya et al. 2011).

**Table 1.** Taxa used in this study, along with their corresponding GenBank accession numbers.

| Taxon | Strain/Specimens | GenBank accession numbers | | | |
|---|---|---|---|---|---|
| | | ITS | LSU | SSU | *rpb2* |
| *Adelosphaeria catenata* | CBS 138679$^{T}$ | KT278721 | KT278707 | KT278692 | KT278743 |
| *Anapleurothecium botulisporum* | FMR 11490$^{T}$ | KY853423 | KY853483 | – | – |
| *Canalisporium exiguum* | SS 00809 | GQ390296 | GQ390281 | GQ390266 | HQ446436 |
| *Canalisporium grenadoideum* | SS 03615 | – | GQ390267 | GQ390252 | HQ446420 |
| *Canalisporium pulchrum* | SS 03982 | GQ390292 | GQ390277 | GQ390262 | HQ446432 |
| *Coleodictyospora muriformis* | MFLUCC 18-1243$^{T}$ | MW981642 | MW981648 | MW981704 | – |
| *Coleodictyospora muriformis* | MFLUCC 18-1279 | MW981643 | MW981649 | MW981705 | – |
| *Conioscypha lignicola* | CBS 335.93 | – | AY484513 | JQ437439 | JQ429260 |
| *Conioscypha minutispora* | CBS 137253 | – | MH878131 | – | – |
| *Dematipyriforma aquilaria* | CGMCC 3.17268$^{T}$ | KJ138621 | KJ138623 | KJ138622 | – |
| *Dematipyriforma muriformis* | MFLU 21-0146$^{T}$ | OM654773 | OM654770 | – | – |
| *Dematipyriforma nigrospora* | MFLUCC 21-0096$^{T}$ | MZ538524 | MZ538558 | – | – |
| *Dematipyriforma nigrospora* | MFLUCC 21-0097 | MZ538525 | MZ538559 | MZ538574 | MZ567113 |
| *Helicoascotaiwania farinosa* | DAOM 241947 | JQ429145 | JQ429230 | – | – |
| *Helicoascotaiwania lacustris* | CBS 145963$^{T}$ | MN699399 | MN699430 | MN699382 | MN704304 |
| *Helicoascotaiwania lacustris* | CBS 146144 | MN699401 | MN699432 | MN699384 | MN704306 |
| *Melanotrigonum ovale* | CBS 138743$^{T}$ | KT278724 | KT278709 | KT278696 | KT278745 |
| *Melanotrigonum ovale* | CBS 138742 | KT278723 | KT278708 | KT278695 | KT278744 |
| *Monotosporella setosa* | HKUCC 3713 | – | AF132334 | – | – |
| *Neoascotaiwania fusiformis* | MFLU 15-1156$^{T}$ | MG388215 | NG_057114 | – | – |
| *Neoascotaiwania fusiformis* | MFLUCC 15-0625 | – | KX550894 | KX550898 | – |
| *Neomondictys aquatica* | KUNCC 21-10708$^{T}$ | MZ686200 | OK245417 | – | – |
| *Neomonodictys muriformis* | MFLUCC 16-1136$^{T}$ | MN644509 | MN644485 | – | – |
| *Nigrellomyces aquaticus* | GZCC 25-0630$^{T}$ | PV871229 | PV871235 | – | PV872880 |
| *Nigrellomyces aquaticus* | GZCC 25-0631 | PV871230 | PV871236 | – | PV872881 |
| *Obliquifusoideum guttulatum* | MFLUCC 18-1233$^{T}$ | MW981645 | MW981650 | MW981706 | – |
| *Obliquifusoideum triseptatum* | CGMCC 3.27014$^{T}$ | PP445243 | PP049503 | PP049521 | PP068779 |
| *Phaeoisaria motuoensis* | KUNCC 10410T | OP626333 | OQ947034 | OQ947036 | – |
| *Phaeoisaria motuoensis* | KUNCC 10450 | OQ947032 | OQ947035 | OQ947037 | – |
| *Phaeoisaria obovata* | CGMCC 3.27015$^{T}$ | PP049488 | PP049504 | PP049522 | PP068788 |
| *Phaeoisaria obovata* | KUNCC 23-15598 | PP049489 | PP049505 | PP049523 | PP068784 |
| *Phaeoisaria sedimenticola* | CGMCC3.14949$^{T}$ | JQ074237 | JQ031561 | – | – |
| *Phaeoisaria sedimenticola* | KUNCC 23-14648 | PP049490 | PP049506 | PP049524 | PP068783 |
| *Phaeoisaria synnematica* | NFCCI 4479$^{T}$ | MK391494 | MK391492 | – | – |
| *Phaeoisaria synnematica* | KUNCC 23-16619 | PP049493 | PP049509 | PP049527 | PP068787 |
| *Phragmocephala stemphylioides* | KAS 4277 | KT278730 | KT278717 | – | – |
| *Pleurotheciella aquatica* | MFLUCC 17-0464$^{T}$ | MF399236 | MF399253 | MF399220 | MF401405 |
| *Pleurotheciella brachyspora* | CGMCC 3.25435$^{T}$ | OR589321 | OR600969 | PP049532 | PP068773 |
| *Pleurotheciella fusiformis* | MFLUCC 17-0113$^{T}$ | MF399233 | MF399250 | MF399218 | MF401403 |
| *Pleurotheciella fusiformis* | MFLUCC 17-0115 | MF399232 | MF399249 | MF399217 | MF401402 |
| *Pleurotheciella guttulata* | KUMCC 15-0442 | MF399239 | MF399256 | MF399222 | MF401408 |
| *Pleurotheciella guttulata* | KUMCC 15-0296$^{T}$ | MF399240 | MF399257 | MF399223 | MF401409 |
| *Pleurotheciella longidenticulata* | CGMCC 3.27018$^{T}$ | PP049496 | PP049513 | PP049531 | PP068776 |
| *Pleurothecium aquaticum* | MFLUCC 17-1331$^{T}$ | MF399245 | MF399263 | – | – |
| *Pleurothecium aquaticum* | MFLUCC 21-0148 | OM654775 | OM654772 | OM654807 | – |

| Taxon | Strain/Specimens | GenBank accession numbers | | | |
|---|---|---|---|---|---|
| | | ITS | LSU | SSU | *rpb2* |
| *Pleurothecium aquisubtropicum* | GZCC 21-0670$^{T}$ | OM339436 | OM339433 | – | – |
| *Pleurothecium pisiformis* | KUNCC 24-19085$^{T}$ | PV264837 | PV264846 | PV335238 | – |
| *Pleurothecium pulneyense* | MFLUCC 16-1293 | – | MF399262 | MF399228 | MF401414 |
| *Pleurothecium semifecundum* | CBS 131482 | JQ429158 | JQ429239 | JQ429253 | – |
| *Pleurothecium semifecundum* | CBS 131271$^{T}$ | JQ429159 | JQ429240 | JQ429254 | JQ429270 |
| *Pseudosaprodesmium cocois* | MFLU 23-0225$^{T}$ | OR438401 | OR438864 | OR458363 | – |
| *Pseudosaprodesmium cocois* | GZAAS 23-0588 | OR438402 | OR438865 | OR458364 | – |
| *Rhexoacrodictys fimicola* | HMAS 43690 | KU999957 | KX033550 | KX033519 | – |
| *Rhexoacrodictys fimicola* | HMAS 47737 | KU999960 | KX033553 | KX033522 | – |
| *Rhexoacrodictys fimicola* | MFLUCC 18-0340 | OM654774 | OM654771 | OM654806 | – |
| *Rhexoacrodictys melanospora* | KUNCC 22-12406$^{T}$ | OP168085 | OP168087 | OP168088 | OP208807 |
| *Rhexoacrodictys melanospora* | KUNCC 22-12411 | OP168093 | OP168101 | OP168099 | OP208808 |
| *Saprodesmium dematiosporium* | KUMCC 18-0059$^{T}$ | MW981646 | MW981647 | MW981707 | – |
| *Sterigmatobotrys macrocarpa* | PRM 915682 | JQ429153 | GU017317 | JQ429255 | – |
| *Sterigmatobotrys rudis* | DAOM 229838 | JQ429152 | JQ429241 | JQ429256 | JQ429272 |

Note: "$^{T}$" indicates ex-type strains. Newly generated sequences are in bold black. "-" indicates the unavailable data in GenBank.

Maximum likelihood (ML) analysis was conducted using the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) based on Bayesian Information Criteria (BIC) (Nguyen et al. 2015). The substitution model was automatically selected by the server. Bayesian inference (BI) analysis was performed using MrBayes on XSEDE (3.2.7a) via CIPRES Science Gateway (Stamatakis 2014). The aligned FASTA file was converted to NEXUS format using AliView (Larsson 2014). The best-fit evolutionary model for each dataset was determined using MrModeltest v. 2.3.10 (Nylander 2004). The GTR+G+I substitution model was selected for LSU, ITS, and *rpb2*, whereas the K80+I+G model was chosen for SSU. Posterior probabilities (BYPP) were determined based on Bayesian Markov chain Monte Carlo (BMCMC) sampling (Huelsenbeck and Ronquist 2001). Four simultaneous Markov chains were run for 10,000,000 generations, and trees were sampled every 1,000 generations. The burn-in phase was set at 25%, and the remaining trees were used to calculate posterior probabilities.

Phylogenetic trees were visualized using FigTree v. 1.4.4 and further edited in PowerPoint. The photo plate was prepared using Adobe Photoshop CS6 software (Adobe Systems, USA).

## Phylogenetic analysis results

The single-locus and multi-locus phylogenetic analyses (LSU, ITS, SSU, and *rpb2*) were implemented to elucidate the phylogenetic position of the two new strains. The concatenated sequence matrix comprised 3,102 characters (LSU: 1–826, ITS: 827–1,371, SSU: 1,372–2,143, and *rpb2*: 2,144–3,102) across 60 taxa. Maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted on the concatenated datasets of LSU, ITS, SSU, and *rpb2*, both yielding similar tree topologies. Fig. 1 presents the best-scoring ML tree, which had a final log-likelihood value of −25777.951173.

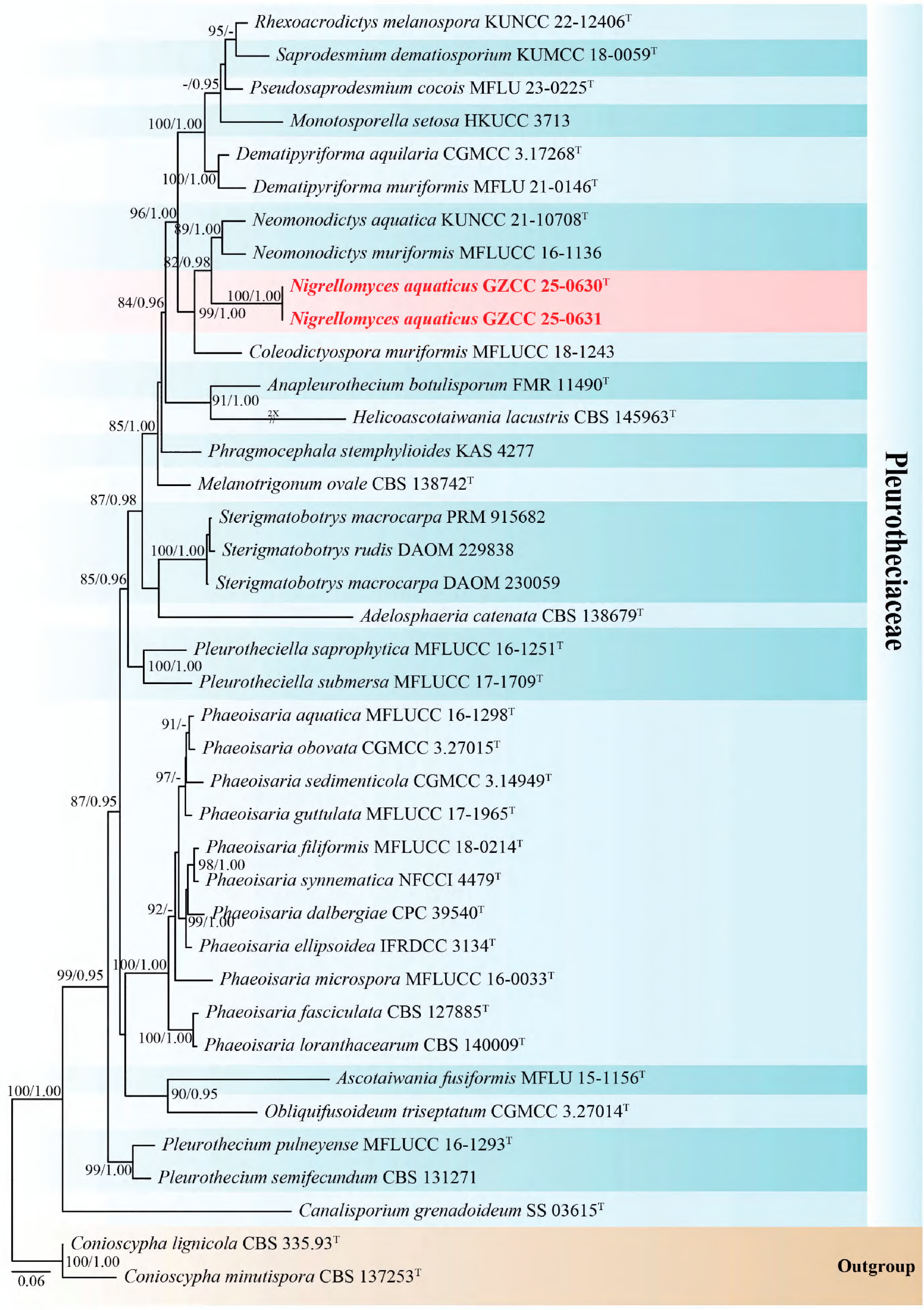


**Figure 1.** Phylogenetic tree generated from ML analysis based on the combined LSU, ITS, SSU, and *rpb*2 sequence data. Bootstrap support values for maximum likelihood greater than 75% (ML left) and Bayesian posterior probabilities ≥ 0.95 (BYPP right) are shown at the nodes. The tree was rooted with *Conioscypha lignicola* (CBS 335.93) and *C. minutispora* (CBS 137253). Ex-type strains are denoted with "T," and newly isolated strains are in bold red fonts.

Based on the multi-gene phylogenetic tree (Fig. 1), our collections represent a new genus and a novel species within the family Pleurotheciaceae (Pleurotheciales, Sordariomycetes). Our isolates (GZCC 25-0630 and GZCC 25-0631) formed a sister clade to the clade comprising *Neomonodictys aquatica* (KUNCC 21-10708) and *N. muriformis* (MFLUCC 16-1136) with weak support.

## Taxonomy

***Nigrellomyces* W.M. Zhang & Q.Y. Feng, gen. nov.**
MycoBank No: 904235

**Etymology.** "*Nigrellomyces*" refers to the small, dark-colored conidia characteristic of this genus.

**Description.** ***Saprobic*** on decaying wood submerged in a freshwater stream. **Asexual morph:** ***Colonies*** superficial, effuse, hairy, brown to black, scattered, with glistening conidial masses at apex. ***Mycelium*** partly superficial, partly immersed, consisting of branched, septate, smooth, smooth-walled, hyaline to pale brown hyphae. ***Conidiophores*** macronematous, mononematous, solitary, erect, unbranched, septate, straight or flexuous, cylindrical, slightly constricted at septa, dark brown, becoming pale brown to brown towards the apex. ***Conidiogenous cells*** polyblastic, integrated, terminal or intercalary sympodial proliferations, sometimes denticles, cylindrical, reniform, swollen at the top, pale brown to brown. ***Conidia*** acrogenous, subglobose to globose, ovoid to obovoid, or ellipsoidal, aseptate, occasionally forming chains in water, guttulate, subhyaline to brown or black, smooth-walled. **Sexual morph:** Undetermined.

**Type species.** *Nigrellomyces aquaticus* W.M. Zhang & Q.Y. Feng

**Notes.** Morphologically, *Nigrellomyces* can be readily distinguished from other genera in Pleurotheciaceae by its polyblastic, reniform conidiogenous cells with curved apices and subglobose to globose, ovoid to obovoid, or ellipsoidal, aseptate conidia that occasionally form chains in water. Phylogenetically, *Nigrellomyces* forms a distinct clade within Pleurotheciaceae, supporting its recognition as a new genus. Herein, we establish the genus *Nigrellomyces* to accommodate a new species, *N. aquaticus*, which is designated as the type species based on both molecular evidence and its distinctive conidial morphology.

***Nigrellomyces aquaticus* W.M. Zhang & Q.Y. Feng, sp. nov.**
MycoBank No: 904236
Fig. 2

**Etymology.** "*aquaticus*" refers to the aquatic habitat of this fungus.

**Holotype.** GZAAS 25-0660

**Description.** ***Saprobic*** on decaying wood submerged in a freshwater stream. **Asexual morph:** ***Colonies*** superficial, effuse, hairy, brown to black, scattered, with glistening conidial masses at apex. ***Mycelium*** partly superficial, partly immersed, consisting of branched, septate, smooth, smooth-walled, hyaline to pale brown hyphae. ***Conidiophores*** macronematous, mononematous, solitary, erect, unbranched, multi-septate, straight, or flexuous, cylindrical, slightly constricted at septa, 146–364 × 4.5–8 µm $\bar{x}$ = 224 × 5.8 µm, n = 25), dark

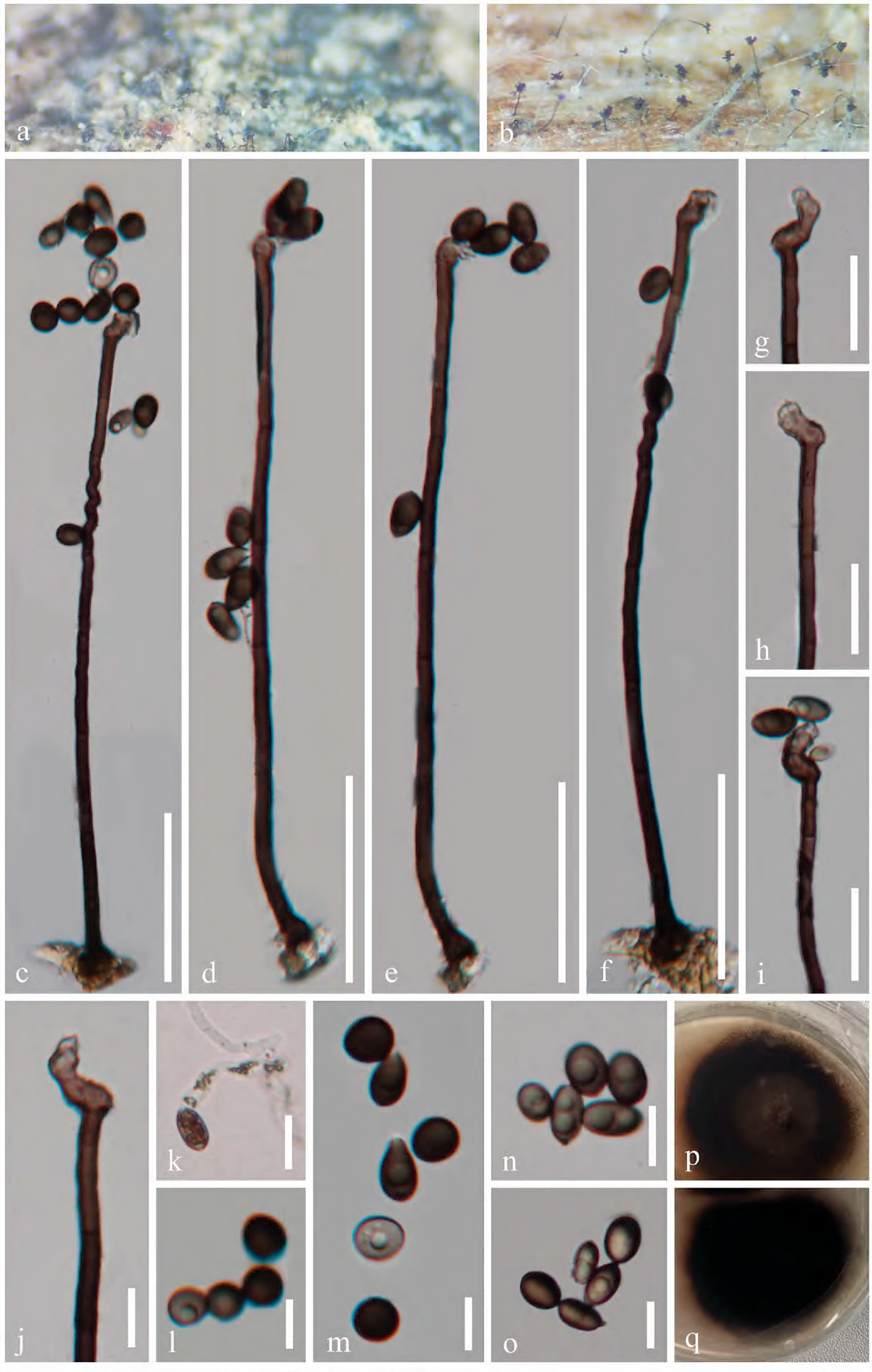


**Figure 2.** *Nigrellomyces aquaticus* (GZAAS 25-0660, holotype). **a, b**. Colonies on the host surface; **c–f**. Conidiophores, conidiogenous cells, and conidia; **g–h**. Conidiogenous cells; **i-j**. Conidiogenous cells and conidia; **k**. Germinated conidium; **l–o**. Conidia; **p, q**. Colonies on PDA from above and below after 41 days of incubation at room temperature. Scale bars: 60 μm (**d–f**); 50 μm (**c**); 20 μm (**g–j**); 10 μm (**k–o**).

brown, becoming pale brown to brown towards the apex. ***Conidiogenous cells*** polyblastic, integrated, terminal or intercalary, sympodial proliferations, sometimes denticles, cylindrical, reniform, curved, and swollen at the apex, 8.5–28 × 4–6 µm $\bar{x}$ = 20 × 5.2 µm, n = 25), pale brown to brown. ***Conidia*** acrogenous, subglobose to globose, ovoid to obovoid, or ellipsoidal, aseptate, occasionally forming chains in water, guttulate, 7.5–12.5 × 5–8.5 µm $\bar{x}$ = 8.9 × 7 µm, n = 25), light brown or black, smooth-walled, sometimes have small apiculus at the base. **Sexual morph:** Undetermined.

**Culture characteristics.** Conidia germinate on PDA within 17 hours, producing germ tubes from the conidial body. Colonies on PDA are irregular with a flat surface and undulate margin, reaching 38 cm in diameter after 41 days at room temperature (approximately 25 °C), and are brown to dark brown on both the surface and reverse sides.

**Material examined.** CHINA • Guizhou Province, Guiyang City, Baiyun District, Changpo Ling National Forest Park, on rotting wood in a freshwater habitat, 15 March 2025, Wang-Ming Zhang, QX13 (GZAAS 25-0660, holotype), ex-type living cultures GZCC 25-0630; • Ibid., QX13.1 (GZAAS 25-0661, paratype), living culture GZCC 25-0631.

**Notes.** In our phylogenetic tree (Fig. 1), isolates (GZCC 25-0630 and GZCC 25-0631) formed a sister clade to *Neomonodictys aquatica* (KUNCC 21-10708) and *N. muriformis* (MFLUCC 16-1136) with weak support. *Nigrellomyces aquaticus* (GZAAS 25-0660) can be distinguished from *Neomonodictys aquatica* (KUN-HKAS 115806) and *N. muriformis* (MFLU 17-1178) by its longer conidiophores, elongated, polyblastic conidiogenous cells, and aseptate conidia (Hyde et al. 2020; Huang et al. 2022). Moreover, base pair comparison of *Nigrellomyces aquaticus* (GZCC 25-0630) and *N. muriformis* (MFLUCC 16-1136) shows 96/588 bp differences in ITS (16.3%, gaps 37 bp) and 22/829 bp differences in LSU (2.7%, gap one bp). Therefore, based on multigene phylogenetic analysis and morphological differences, we introduce *Nigrellomyces aquaticus* as a novel genus and species.

## Discussion

The newly introduced genus *Nigrellomyces* shares morphological similarities with *Cordana*, including brown, septate conidiophores; polyblastic, swollen conidiogenous cells; and brown, obovoid to ellipsoidal conidia (Hughes 1955; Hernández-Restrepo et al. 2014; Hernandez Restrepo et al. 2015; Kuo et al. 2024; Xu et al. 2024b). However, *Nigrellomyces* differs from *Cordana* in several key features. Notably, its conidiogenous cells are curved at the apex and occasionally bear denticles, while its conidia are aseptate, in contrast to the typically septate conidia of *Cordana* (Hughes 1955; Hernández-Restrepo et al. 2014; Hernandez Restrepo et al. 2015; Kuo et al. 2024; Xu et al. 2024b). In addition to these morphological distinctions, the two genera are phylogenetically unrelated. *Cordana* is classified within the family Cordanaceae (Coniochaetales, Sordariomycetidae), whereas *Nigrellomyces* is placed in Pleurotheciaceae (Pleurotheciales, Savoryellomycetidae). Our multilocus phylogenetic analysis revealed that *Nigrellomyces* forms a monophyletic clade with strong support, positioned as a sister group to *Neomonodictys* within Pleurotheciaceae. Morphologically,

*Nigrellomyces* is distinct from other genera in the family. While most members of Pleurotheciaceae exhibit acrodictys-like (Yanna and Hyde 2002; Sadowski et al. 2012), helicoön-like (Dayarathne et al. 2019; Réblová et al. 2020), monodictys-like (Hyde et al. 2020), and dactylaria-like (Réblová et al. 2016; Luo et al. 2018a) asexual morphs, *Nigrellomyces* displays unique cordana-like characters. Given its morphological and phylogenetic uniqueness, *Nigrellomyces* is established here as a new genus to accommodate this newly discovered taxon.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

### Use of AI

No use of AI was reported.

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### Author contributions

Writing - original draft: XS, WZ. Writing - review and editing: QF, XZ, WX, JL.

### Data availability

All of the data that support the findings of this study are available in the main text.

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